

## Nanostructured Functionally Graded Metalloceramic Biomaterials

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In this highlight, we discuss processing, mechanical properties, and mesenchymal stem cell adhesion/spreading on two types of biomaterials: functionally graded plasma-nitrided Ti-6Al-4V and nanostructured porous polymeric scaffolds made by electrospinning method. The objectives in this project are to improve wear and osseointegration of load-bearing implants (e.g. hip and knee) and to develop new bone-tissue engineering strategies, respectively.

**Plasma-Nitrided Ti-6Al-4V:** Improved methods to increase surface hardness of metallic biomedical implants are being developed in an effort to minimize the formation of wear debris particles that cause local pain and inflammation. However, for many implant surface treatments; there is a risk of film delamination due to the mismatch of mechanical properties between the hard surface and the softer underlying metal. We have modified the surface of titanium alloy (Ti-6Al-4V) using microwave plasma chemical vapor deposition (MPCVD) to induce titanium nitride formation by nitrogen diffusion and subsurface implantation. The result is a gradual transition from a titanium nitride surface to the bulk titanium alloy, without a sharp interface that could otherwise lead to delamination. Compared to conventional ion-beam methods which implant nitrogen ions only to about a few hundred nanometers depth, we can achieve deeper nitriding (greater than 1 micron) with higher resulting hardness at a given depth (see Fig. 1). We demonstrate that vitronectin adsorption, as well as the adhesion and spreading of human mesenchymal stem cells (hMSCs) to plasma nitrided titanium is equivalent to that of Ti-6Al-4V (see Figs. 2 and 3); while hardness is improved 3-4 fold. We observe indistinguishable morphology of hMSCs cultured for 1

hour on uncoated control Ti-6Al-4V vs. plasma nitrided Ti-6Al-4V surfaces. Similarly, no differences in morphology were apparent for cells adherent to serum-coated Ti-6Al-4V or plasma nitrided Ti-6Al-4V substrates. We also examined the morphology of cells at 24 hours following cell seeding. These experiments revealed that, as before, there were no distinguishable differences in the morphology of cells adherent to the plasma-nitrided vs. control Ti-6Al-4V samples, regardless of whether surfaces were uncoated or pre-coated with serum. Not surprisingly, however, substantially more cells were apparent on serum-coated substrates, which likely reflect increased cell proliferation and/or survival. Our data indicate that cells respond equally well to plasma nitrided

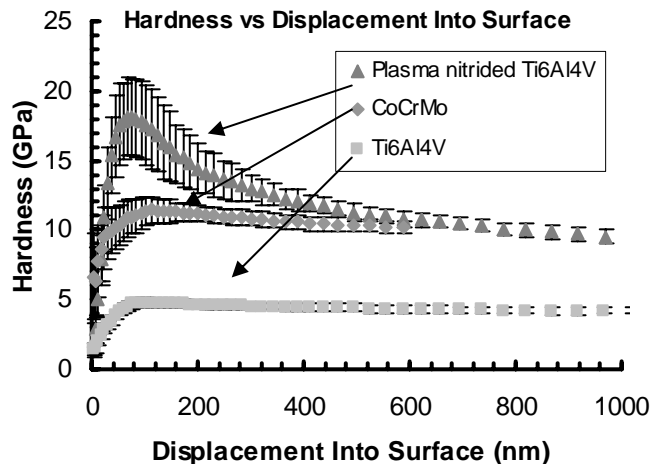


Fig. 1. Hardness of microwave plasma nitrided Ti-6Al-4V, CoCrMo, and bare Ti-6Al-4V as measured by nanoindentation. There is sufficient nitrogen reaction with titanium to produce a hardness that is twice that of bulk alloy at 1 micron depth.

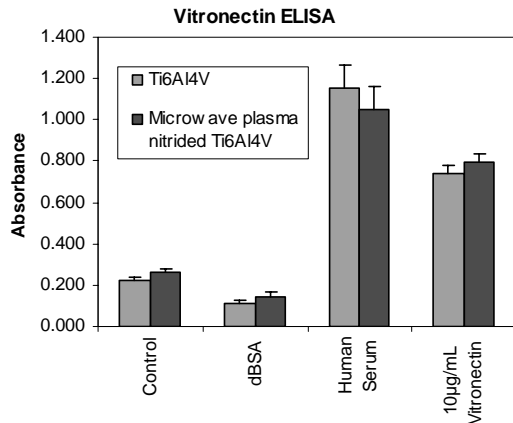


Fig. 2. Vitronectin ELISA. Substrates were coated with human serum, purified human vitronectin, denatured BSA, or left uncoated (control). There was no statistical difference between the amount of vitronectin associated with plasma nitrided vs. control Ti-6Al-4V surfaces.

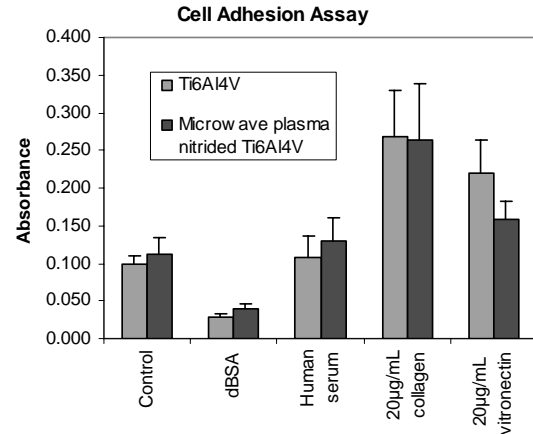


Fig. 3. hMSC adhesion assay. Substrates were coated with human serum, vitronectin, collagen I, denatured BSA or left uncoated (control). There was no statistical difference between the number of hMSCs that adhere to plasma nitrided vs. control Ti-6Al-4V, regardless of the surface treatment.

and control Ti-6Al-4V surfaces. These *in vitro* results suggest that the functionally graded plasma nitriding technique has the potential to reduce wear, and the resulting debris particle release, of biomedical implants without compromising osseointegration, thus minimizing the possibility of implant loosening over time.

**Nanostructured porous polymeric scaffolds for tissue engineering:** An ideal 3D-scaffold for tissue engineering requires similarity to natural counter-parts in terms of chemistry, physical nano-feature and bio-performance. In-vitro seeding and attachment onto a nano-structured scaffold can allow excellent cell proliferation, migration, and differentiation of the cells into specific tissue while secreting the extra cellular matrix (ECM) components required for continuing tissue growth. Collagen is a major natural ECM component, and possesses a fibrous structure with fiber bundles varying in diameter from 50-500 nm. A nano-fibrous composite of synthetic polymer or biopolymer with nano-hydroxyapatite, that mimics the nano-scale features of ECM, could be a promising system for next generation scaffolds for bone tissue engineering. The electrospun bioactive nanocomposite nanofibers of polycaprolactone and collagen (type I) have been fabricated into different 3D geometry (sheet, tube and rope) via electrostatic-co-spinning. NanoHA loading varied from 0% to 20 % by weight to get improved mechanical properties. SEM analyses (Figs. 4-6) showed that scaffolds have porous nanofibrous non-woven structures with a well interconnectivity of pore network structure, which is essential for exchange nutrients and metabolic wastes to facilitate proper cellular in-growth. The majority of fibers have diameters in the range of 350 nm to 650 nm, depending on the composition. The mechanical properties increase with increase of nanoHA content in the fibrous matrix. In the case of collagen/nanoHA fibrous composites, further chemical cross-linking of collagen improved the mechanical properties of the fibers. Significant differences in morphology and cell number were observed when MSCs are cultured on electrospun polycaprolactone (PCL) fibrous scaffolds

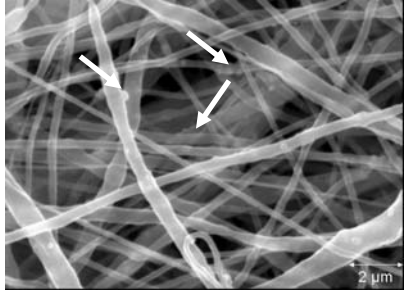


Fig.4 SEM of PCL/nanoHA composite scaffolds. As nanoHA content increases, roughness of fiber increases. Arrows show nanoHA particles on the surface of fibers.

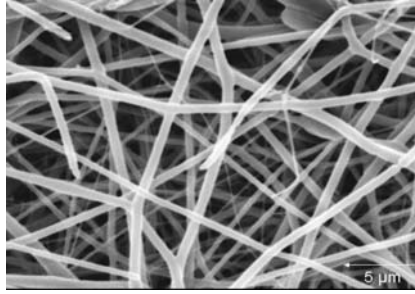


Fig.5 SEM of electrospun collagen scaffolds before cross-linking.

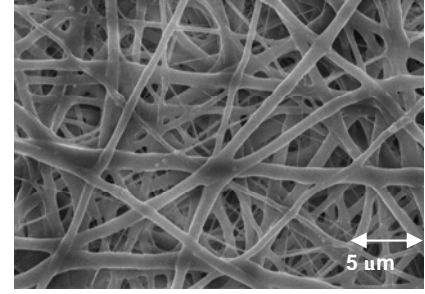


Fig. 6 SEM of nanofibrous collagen/hydroxyapatite composite scaffolds after cross-linking. Cross-linking increases the fiber diameters and inter-fiber binding at intersection of fibers.

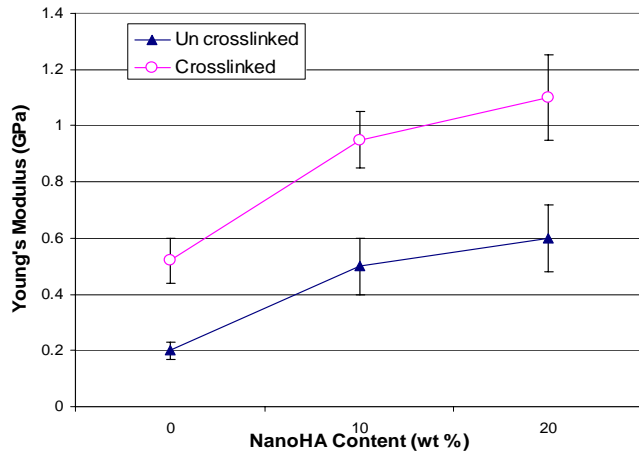


Fig. 7 Young's modulus of collagen/nanoHA composites vs. nanoHA content (wt%). As nanoHA content increases the modulus and hardness (by nanoindentation) increase. Chemical cross-linking of collagen fiber further increases the modulus.

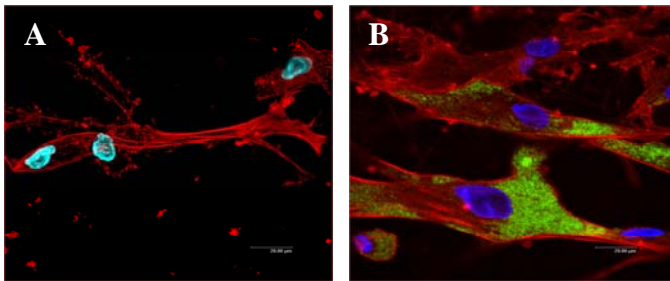


Fig. 8 Human MSCs seeded onto fibrous scaffolds composed of (A) 100% PCL and (B) 80% PCL and 20% HA. The HA caused the cells to develop a wider morphology and green autofluorescence.

versus fibrous scaffold composites composed of 80% PCL and 20% hydroxyapatite (HA) composites composed of 80% PCL and 20% hydroxyapatite (HA) (Fig. 2).

References

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# 2005 NSF NIRT RET Participants



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